REMARKS

REJECTIONS UNDER 35 U.S.C. § 112

The examiner rejects claim 17 under 35USC 112, for failing to particularly point out and distinctly claim the subject matter as it recites "simultaneously prepared and purified". Claim 17 has been amended, and the amended claim renders the rejection of the Examiner overcomes said the rejection. Not to acquiesce rejection but to facilitate prosecution, applicant respectfully requests the examiner to withdraw the rejection under 35 USC 112 with respect to claim 17.

REJECTIONS UNDER 35 U.S.C. § 103

Examiner rejects claims 1-4, 6, 8, 9, 14-16 and 18 under 35 USC 103 as allegedly being obvious over Lakshmi et al. (Vaccine, 2000), Nielsen et al. (WO2003/050274), da Costa et al. (biotechology techniques, 1995) and Bitter et al. (Journal of Medicinal Virology, 1988). Applicant respectfully traverses the rejections of the examiner.

To establish a prima facie case of obviousness, the cited references in combination or individually must teach or suggest each and every claim limitation. As described in detail below, the combination of Lakshmi, Nielsen, da Costa and Bitter does not teach each and every element of the claims, as currently amended. Moreover, the cited references teach away from the invention as presently claimed.

THE CITED REFERENCES DO NOT TEACH OR SUGGEST EVERY ELEMENT OF THE PRESENTLY-AMENDED CLAIMS.

Lakshmi et al. is directed to evaluate the immunogenicity and seroprotection of recombinant Hepatitis B vaccine. Although it includes a flowchart of production of the recombinant Hepatitis B vaccine in yeast, it does not disclose anything specifically about process of purification of the recombinant surface of Hepatitis B antigen to be used in the intended vaccine. Merely it discloses hydrophobic interactions in one of its steps in the flowchart, wherein any details of

such purification is absent in Lakshmi et al. Moreover, the applicant also recognizes that such production of protein molecules using prokaryotic and/or eukaryotic cell systems is already known in the art in paragraph 002 of the US pre-grant publication US 2007/0154880 A1. "Use of prokaryotic and eukaryotic cell systems for the production of various therapeutic protein molecules is a common method in present day Biotechnology. In this process, the protein of interest is expressed in the said cell system by suitably engineering the molecular genetics of the expression system to incorporate a plasmid to promote the production of the desired proteins when suitably induced during the growth of the cells.", Rather the current application teaches a method to purify proteins or antigens with high concentration and negligible loss of biological activity, obtained through recombinant cells like yeast for instance in this particular case. Examiner also admits that Lakshmi et al. does not teach that cells are lysed, forming an insoluble matrix with divalent salt along with counter ions of chloride or acetate (Office Action page 5).

Further, Nielsen et al does not overcome these shortcomings of Lakshmi et al. Nielsen et al. teaches a method of production of crystallized or amorphous metabolite suspension from a fermentation broth. As it is evident from the Title, Technical Field, Summary, as well as the Detailed Description of Nielsen (PCT/DK02/00821), that it teaches recovery of crystal or amorphous particles of metabolite suspension from a fermentation broth. The objective of Nielsen is entirely different from the present invention. Nielsen is silent about any biological activity of the metabolite suspensions those are crystallized from the fermentation broth, whereas the present application deals with purification of the recombinant antiviral antigens and therapeutic proteins in terms of attaining maximum biological activity at higher concentrations and filtering out unwanted contaminants. Nielsen teaches percentage crystal structure of the end products so obtained only.

The metabolite of interest as defined in Nielsen cannot be said to include recombinant viral antigens to be used in vaccines, or therapeutic proteins. Even, in lines 25-27 of Nielsen at page 1 teaches that, the metabolite may also be a therapeutic protein *(emphasis added)*, "The metabolite may also be **a protein** *(emphasis added)*, e.g. **a therapeutic protein (emphasis added)** such as insulin or an enzyme." Therefore Nielsen does not teach any method of purification of any therapeutic proteins under the presently-amended claims in the present invention.

Further, the technology involved in Nielsen is related to recovery directly from the Fermentation broth. The present invention relates to purification of the intended antigen or therapeutic protein from the **cell lysate.** Cell Lysate is obtained after breaking the cells through lysis. Lysis is performed subsequent to fermentation from the fermentation broth, followed by centrifugation to obtain a Cell Lysate. The inventive process of the present application does not purify the intended antigens and/or therapeutic proteins from the fermentation broth directly. It involves additional steps to obtain a Cell Lysate from the fermentation broth. Although the process involved in Nielsen tells about adding divalent salts for coagulation of having counter ions of chloride, such coagulation is from the fermentation broth whereas the insoluble matrix formed under the present invention by addition of divalent ionic salts along with counter ions is from the cell lysate obtained after serial spinning of the fermentation broth. Therefore application of salts for the purpose of coagulation to the fermentation broth cannot be said to be obvious with respect to addition of ionic compounds to the Cell Lysate intended to purify the proteins present in the Cell Lysate, for the basic reason that, crystallization is a different phenomena as compared to purification of antiviral antigens and therapeutic proteins altogether. Coagulation is a process of extraction of the desired protein present in the fermentation broth, whereas the present application teaches purification of the extracted protein to achieve maximum biological activity.

The shortcomings of Nielsen and Lakshmi are not taught by da Costa et al. da Costa teaches desorption of yeast culture supernatant with TRIS buffer at a pH of 9.0. da Costa do not teach adsorption of any insoluble matrix in presence of divalent cations and counter ions. The present application also teaches that desorption process must be done after capturing of antigens and/or proteins in divalent cations along with counter ions. The present application use either TRIS buffer specifically at pH range of 8.0-8.5 or TRIS and EDTA buffer at pH of 7.0 to 8.0, whereas da Costa teaches desorption at a higher pH of 9 only with TRIS buffer. Since, all these limitations including treatment with divalent cations of the insoluble matrix and subsequent treatment with TRIS and EDTA (pH 7 to 8) or only TRIS buffer (pH 9) are absent in da Costa, it is not obvious to apply the teachings of da Costa to arrive the present invention. Furthermore, the general conditions of desorption is also absent in da Costa, since da Costa does not teaches use of TRIS and EDTA buffer at a pH from 6 to 8. The present invention undergoes repeated

desorption process with a combination of two buffers, which is different to that of the teachings of da Costa (only TRIS at pH more than 9). Therefore, the applicant opines that the rejection under optimization of general conditions as put forward by the Examiner is also not applicable in the present case. Also, with due regard to the views of the Examiner and not to acquiesce rejection, the applicant humbly submits that, da Costa was not cited in the International Search Report of the PCT application pertaining to the present application which also additionally clarifies that the cited prior art of da Costa does not render obvious the present application.

Bitter et al. teaches that yeast cells were subjected to lyse the cells through agitation with glass beads to recover the resulting supernatant. The applicant humbly submits that, the present invention does not claim specifically any method to lyse the cells. Lysis of cells is already an established process known in the art. In the present invention, lysis is the primary step to start the claimed invention of purification of antigen and bio-therapeutic proteins so that the person skilled in the art reading the invention understands the invention in a better manner. Not to acquiesce to examiner's rejection but to facilitate prosecution, the applicant has currently amended the claims 1 and 3 and hence the rejection of the Examiner is rendered moot with respect to Bitter et al. Also, with due regard to the views of the Examiner, the applicant humbly submits that, Bitter et al. was not cited in the International Search Report of the PCT application pertaining to the present application which also additionally clarifies that the cited prior art of da Costa does not render obvious the present application.

In light of the above arguments mentioned above, applicant requests the Examiner to withdraw the rejections under 35 USC § 103 as applied to the cited prior arts of Lakshmi et al., Nielsen et al., da Costa et al., and Bitter et al. over the present application since all the claim limitations of the present inventions are not present in the cited prior arts mentioned by the examiner.

THE CITED REFERENCES TEACH AWAY FROM THE PRESENTLY-AMENDED CLAIMS .

In addition. the disclosures of Laksmi et al., Nielsen et al., da Costa et al., and Bitter et al., teach away from the combination of these references. As pointed out by Nielsen (PCT/DK02/00821)

on page 6 lines 13-19, "It is possible to coagulate and/or flocculate the metabolite fermentation broth so that the crystalline and/or amorphous metabolites are in the right separation zone and thus can be separated in e.g. a centrifuge process into a biomass fraction (with a very low metabolite concentration), the crystalline and/or amorphous metabolite fraction (with a high metabolite concentration) and the **supernatant fraction (with a very low metabolite concentration).**" *(emphasis added on supernatant fraction with a very low metabolite concentration)* Therefore it is evident that the intended metabolite as referred by Nielsen is not present in the supernatant at all, whereas in the present application, the specification clearly mentions that the protein is purified from the supernatant. Reference is made to para **[0041]** of the present specification, "The cell lysate after fermentation is subjected to centrifugation and the insoluble fraction is treated with detergent. **The supernatent after centrifugation was** either **subjected to** Aerosil adsorption and desorption (traditional technology) (table 1) or **to primairy capturing of HBsAg by a batch procedure in which salts of divalent cations such as Calcium, Magnesium and Zinc are added at 0.2% to 10% (w/v) in the presence of phosphates, Chlorides or Acetates to form white insoluble matrix.** The *in situ* formation of the matrix further interact with the antigen and this process of protein capturing is referred as HIMAX technology (table 20). This matrix was separated by centrifugation between 7000 g to 10,000 g and bound antigen was desorbed repeatedly with this buffer of pH 8.5."

Also Table II para [0045] of the specification makes it clear that the supernatant with 84% biological activity (S. No.6) is allowed to adsorption and desorption step of the present invention which retains 80% of the biological activity (S. No.7), from the supernatant.

Due to the above differences in the teachings of Nielsen et al., and the present invention to purify the intended proteins from the supernatant, the skilled artisan would have been dissuaded from performing the claimed invention under the present application as suggested by the examiner. Accordingly, Nielsen teaches away from the combination of references used by the examiner in making this rejection. For this reason also, the examiner is respectfully requested to withdraw the rejections under 35 U.S.C. § 103.

CONCLUSION

Applicant now believes that this amendment complies with 37 CFR § 1.121 and thus requests examination of this Amendment. Applicant respectfully requests that a timely Notice of Allowance be issued in this case.

Respectfully submitted,

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